



Comprehensive approach to a taxonomically complex group - genus *Quercus* (Oak) in the Bulgarian flora

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Genus *Quercus* – often considered as *Crux Botanicorum* among the taxonomists

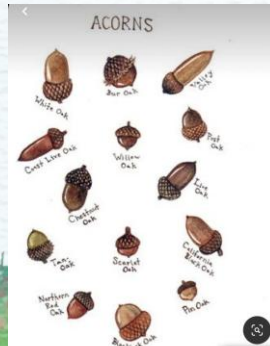
Great diversity

Linnaeus (1753) – 14 species; Loudon (1839) – 150 species;

First half of the 20th century – more than 300 (Ørsted, Schwarz, Camus); currently > 500.

The genus (and especially some sections) possesses almost all peculiarities of a taxonomically complex group (TCG): reticulate evolution, hybridization, overlapping of key traits (continuous variation); species boundaries and species delineation difficult.

Recently – two important works: Denk et al. (2017) – updated infrageneric classification; Kremer & Hipp (2020) – Oaks: an evolutionary success story (New Phytol.)



In Bulgaria

Oak forests ~ 24 %

- Velenovsky (1891, 1898) – six species
- Stojanoff & Stefanoff – 8 (9) species
- The last summarization Flora of Bulgaria vol. 3. (Ganchev & Bondev 1966): 16 species (15 numbered), many intraspecific taxa and 8 putative hybrids (based entirely on morphology).
- Since 1966 – occasional changes – adding two new species (*Q. trojana* and *Q. protoroburoides* sp. nova) and dropping out three (Uzunova 1987; Di Pietro et al. 2012 и др.)
- Today, 60 years later, there is an urgent need of critical reassessment of the genus taxonomy



In Bulgaria

Controversial taxa (some of):

Q. pedunculiflora K. Koch

Quercus longipes Steven

Q. erucifolia Steven

Q. mestensis Bond.

Q. dalechampii Ten. (cf. DiPietro et al. 2012)

Q. polycarpa Schur

Q. virgiliana Ten.

Q. brachyphylla Kotschy

Putative hybrids between *Q. petraea* and *Q. pubescens*,
Q. petraea and *Q. frainetto*, *Q. cerris* and *Q. coccifera*.

The Euro+Med PlantBase - the information resource for Euro-Mediterranean

Euro+Med Plantbase integrates and critically evaluates information from Flora Europaea, Med-Checklist, the Flora of Macaronesia, taxonomic and floristic literature. This is complemented by the European taxa of several families taken from the World Checklist and Information Service ILDIS (see [credits](#) for details). By 1st of February 2018 it provides access to the total European flora.

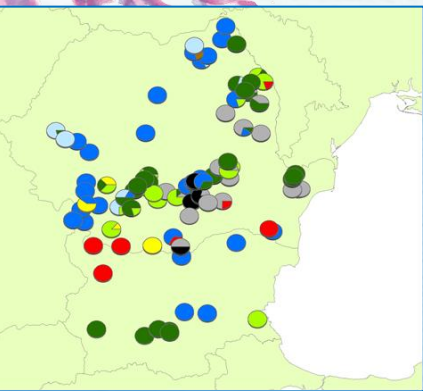
This version of Euro+Med PlantBase is not updated any more. Updates can be found at the [data portal](#).

Details for: *Quercus*

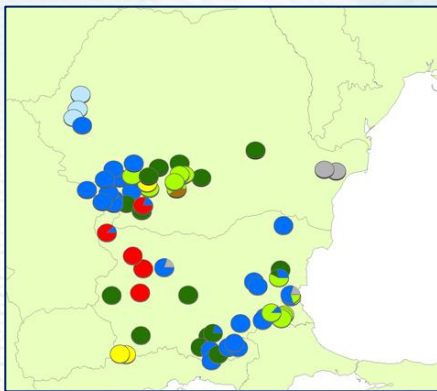
Source:	World Checklist of Selected Plant Families (2010), copyright © The Board of Trustees of the Royal Botanic Gardens, Kew
Name:	<i>Quercus</i> L.
Nomencl. ref.:	<i>Sp. Pl.</i> : 994. 1753
Rank:	Genus
Status:	ACCEPTED
Taxonomy (this taxon is included in):	Regnum - Plantae Divisio - Tracheophyta Subdivisio - Spermatophytina Class - Magnoliopsida Superordo - Rosanae Ordo - Fagales Engl. Familia - Fagaceae Dumort.



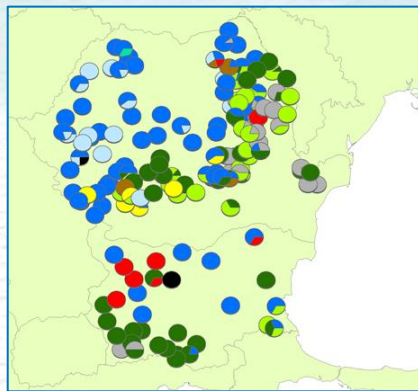
Unique genepool (in most cases)



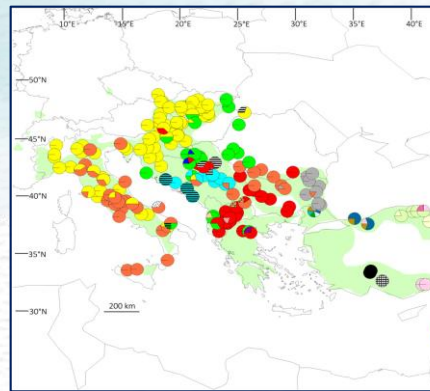
Q. robur



Q. frainetto



Q. petraea



Q. cerris (Bagnoli et al.)



The combined (comprehensive) approach

DNA – barcoding; additional
taxonomic and genetic markers

Traditional (classical) morphology
and morphometry

Metabolomics
(chemosystematics)

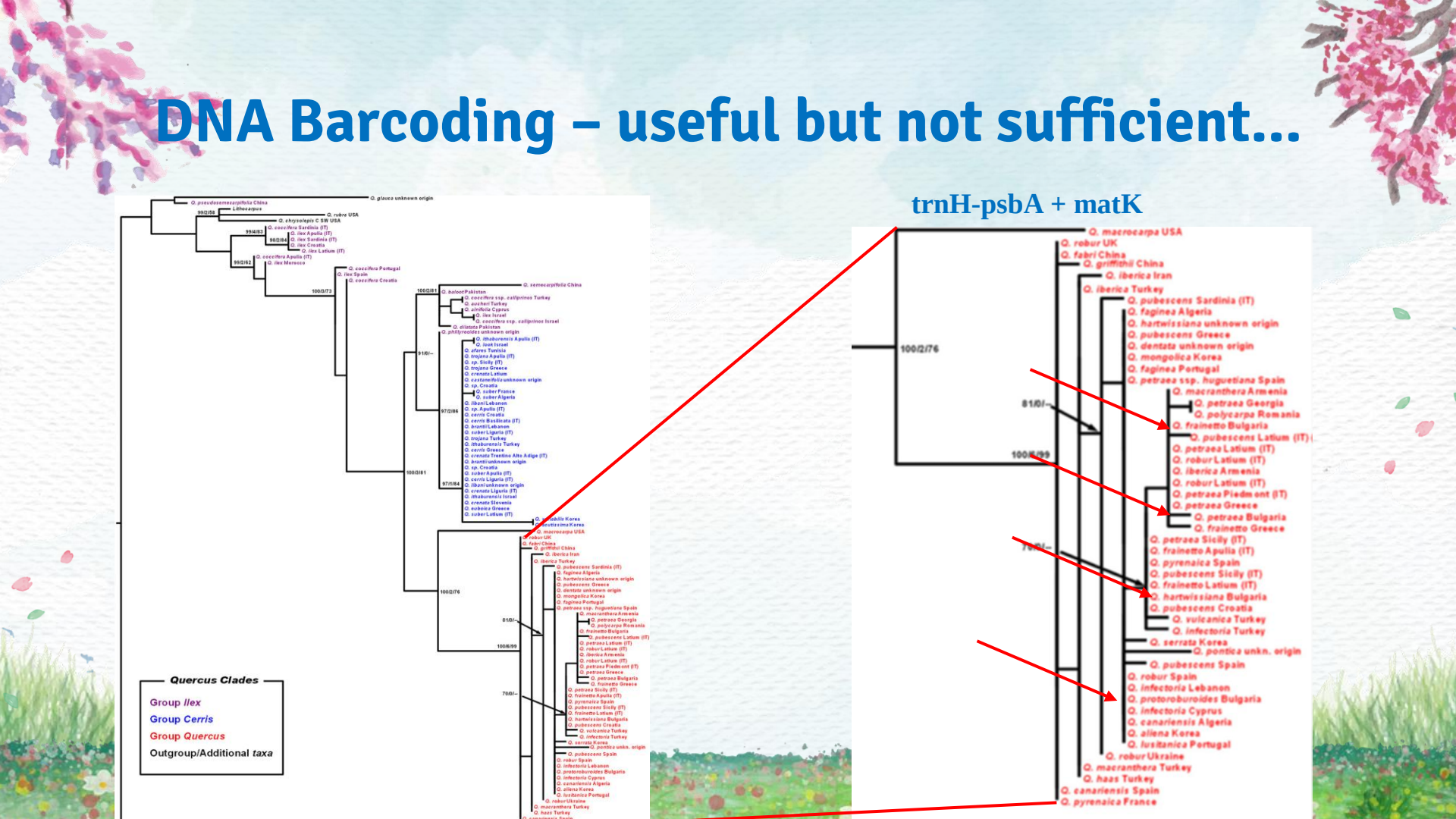
Geometric morphometrics

Pollen morphology

Leaf epidermis morphology

TCG
Case study:
Quercus

the great absentee! – chromosome cytology

[illegible]

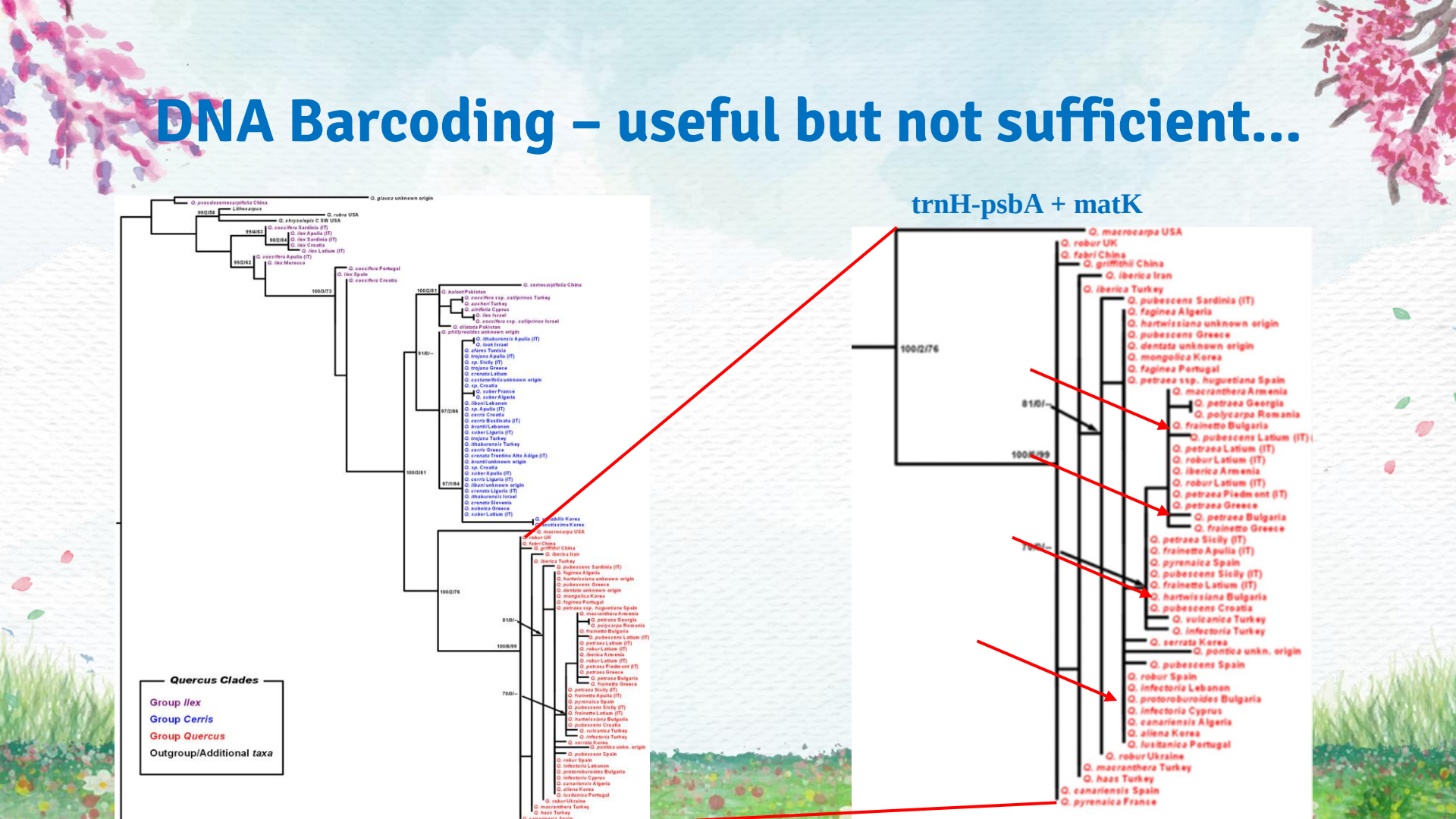
DNA Barcoding – useful but not sufficient...

Quercus Clades

- Group *Ilex*
- Group *Cerris*
- Group *Quercus*
- Outgroup/Additional taxa

trnH-psbA + matK

Phylogenetic trees illustrating the relationships between various *Quercus* species and their geographic origins, based on DNA barcoding data (trnH-psbA + matK). The trees show the distribution of species across different regions, including Europe, Asia, and North America, and highlight the challenges of using DNA barcoding alone for species identification.



DNA barcoding and other markers - further steps

Marker	Type	Length (bp)
<i>trnH-psbA</i>	chloroplast	690
<i>matK</i>	chloroplast	550-580
ITS2	nuclear	363-373
<i>matK</i> + <i>trnH-psbA</i>	chloroplast - combination	1240-1290

- Markers applied to date – good estimate of variation among the groups of species and groups of populations, but insufficient for taxonomic purposes. Genealogy!
- Including new markers and their combinations: *petD* group II intron, *ycf-1*...
- Including chloroplast and nuclear microsatellites for delineation of boundaries of the real biological entities.

Species	<i>trnH-psbA</i> (1)	<i>matK</i> (2)	ITS-2 (3)	Species identification
<i>Q. robur</i>	4	3	4	only in combination
<i>Q. frainetto</i>	4	2	2	No
<i>Q. hartwissiana</i>	2	2	2	No
<i>Q. protoroburoides</i>	1	1	1	No
<i>Q. petraea</i>	4	3	5	2 & 3, combined
<i>Q. polycarpa</i>	1	1	1	3, combined

- Applying more sophisticated bioinformatic methods for documenting the variation in some regions not studied before; comparison with published sequences (Plomion et al. 2015).
- If possible – nuclear ITS sequences and other approaches related to massive genome sequencing.

Geometric morphometrics

Q. hartwissiana – close to Black Sea



Q. protoroburoides – in Rila Mts., 1300-1700 m a.s.l.



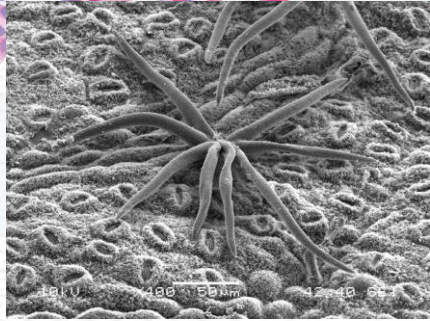
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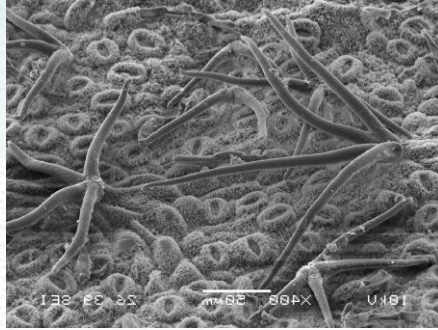
Leaf Morphology, Taxonomy and Geometric

2011

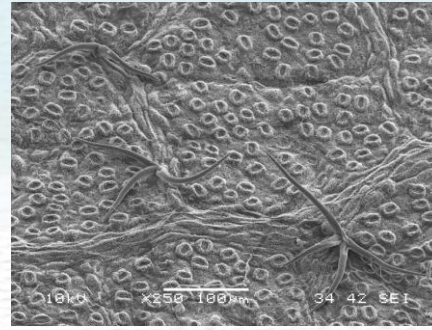
Leaf micro-morphology (SEM study)



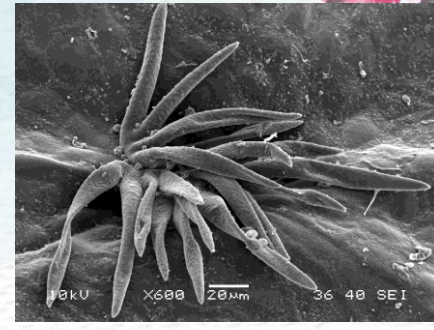
Q. Protoroburoides – 1



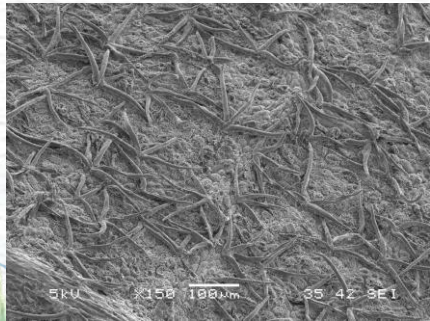
Q. Protoroburoides – 2



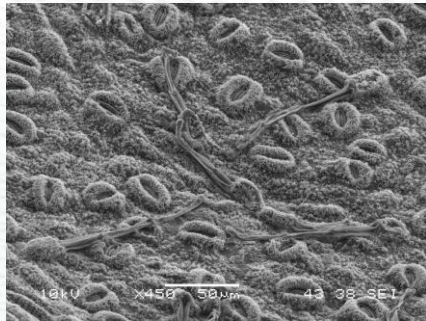
Q. hartwissiana



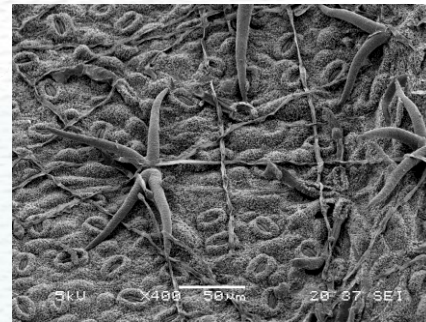
Q. mestensis



Q. pedunculiflora



Q. robur



Q. petraea



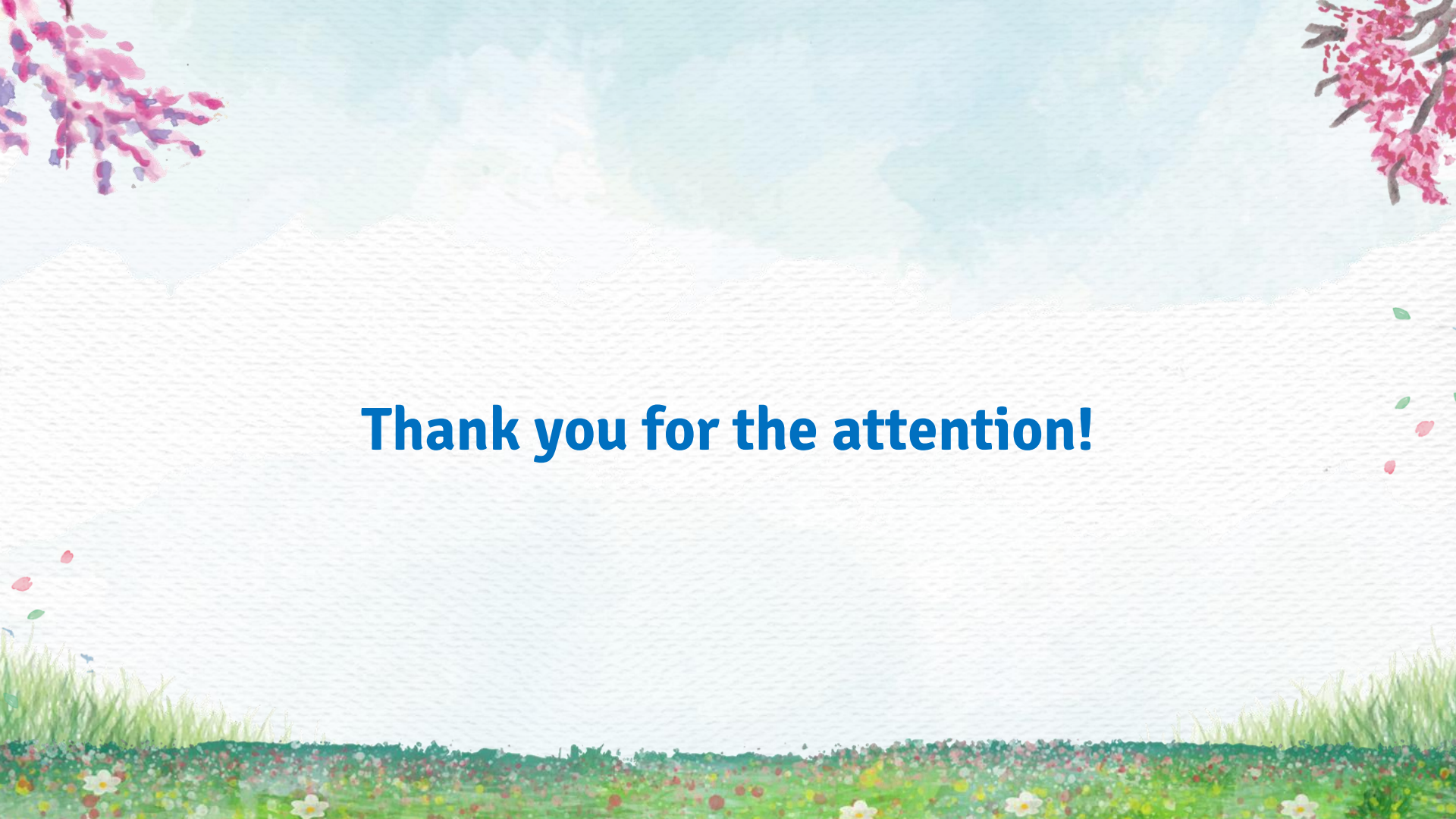
Q. frainetto

Still to be addressed

- **Secondary metabolites** – the preliminary screening study revealed that the variation is higher within species than between species. Hope to find at least quantitative differences at the species level.
- **Ecology** – works in several but not all case studies. *Q. robur* and *Q. petraea* s.l. do not meet; Evident differences between *Q. pedunculiflora* (in most cases in the lowlands) and *Q. mestensis* (800-900 m a.s.l.).
- **Reproductive biology** – relatively uniform among the species of the same subgenus (1-year reproductive cycle in subgenus *Quercus* and 2-year reproductive cycle in subgenus *Cerris*).
- **Combined evaluation** (simplified variant of Omega) – it's a long way to

Acknowledgements

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Thank you for the attention!